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Biodegradation Of Lubricating Oil By Three Species Of Candida

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Abstract

This work examines the biodeterioration of lubricating oil in the presence of aqueous phase by Candida species isolated from used oil, the isolated strains were identified as *Candida petrophilum, Candida lipolytica* and *Candida pulcherrina* , it was found that the yeasts were able to utilize the base oil and it's additives as a sole source of carbon and energy ,the degradation processes was monitored by measuring the total viable count of the cells, dry weight of the biomass and the final pH of the aqueous phase of the medium, also the effect of different temperatures and pH on these isolates were determined. Data of the temperature and pH related biomass yield, showed that the best biodegradation of the lubricating oil was occurred at 25 C degree and at a pH of 5.6 for all the isolates .

الخلاصة

يتضمن العمل الحالي التردى الحيوي لزيت التشحيم بوجود الطور المائي بفعل أنواع من الخمائر عزلت من زيوت مستخدمة، وقد شخصت عزلات الخمائر على أنها*Candida petrophilum , Candida lipolytica , Candida pulcherrina*

ووجد إن الخمائر كانت قادرة على استهلاك زيت الأساس والمضافات كمصدر وحيد للكاربون والطاقة ، وقد تم تحديد عملية التردي من خلال حساب عدد الخلايا الكلي ، الوزن الجاف للكتلة الحية ودالة الحموضة النهائية للطور المـائي كمـا درس تـأثير الدرجات الحرارية المختلفة ودالة الحموضة على العزلات ، واظهرت النتائج المرتبطه بالكتله الحيويه ان افضل تردي حيوي لزيت التشحيم حصل عند درجه حراره 25 م وداله حموضه 5.6 ولجميع العزلات.

Introduction

The interest in petroleum microbiology has gradually developed on a parallel with the increased production of crude oil and the progress in petroleum technology, one of the earliest reports on biodegradation of hydrocarbons was published by Miyoshi (1895) who was observed the utilization of paraffinic wax by *Botrytis cinera* (Mgot,2005), since the earlier work in petroleum microbiology, information about hydrocarbon biodegradation and biosynthesis has been accumulated gradually, this information has been applied in many ways including the microbiological disposal of petroleum wastes which build up in the environment from oil production ,transport , refining and other petroleum products industries, researches have also been directed towards elucidation of the mechanisms of deterioration and to find inhibitors that control the microbial activities associated with contaminated petroleum products (Atlas & Cerniglia , 1995 , Atlas & Bartha ,1992) ,most of the work was carried out using bacteria, however more recently many studies on yeasts have been reported (Duarte ,2001, Eckford & Fedorak , 2002 , Kent & Triplett ,2002) . Lubricating oils are fluid Lubricants used to reduce friction between bearing surfaces, these oils may be mineral oils produced from the distillation of crude oils or synthetic oils composed mainly of esters and methyl silicones , mineral Lubricating oils are basically hydrocarbons containing thousands of different compounds of varying structural types, the major components of these oils are paraffins and naphthenes , but they also contain small amounts of aromatic hydrocarbons especially monocyclic compounds (Magot ,2005), the aim of this work is to isolate and identify the yeasts capable of growth in Lubricating oil and to study their capability of growth over a wide rang of temperature , pH and to examine their possibility to utilize base oil additives.

Materials and Methods

1- Isolation of Microorganisms

Samples of used oil and of associated water bottoms were taken from drain points of tanks usually contained used oils in a sterile bottles , 0.1 ml of it was streaked on malt extract agar, Tryptose blood agar base and meat extract agar and incubated at 30◦c and 48 ◦c for 3 days, selected individual colonies were sub-cultured until pure (Korda, et al. 2007)

2-The ability of the isolates to grow on fresh oil

An inoculums was prepared by suspending the cells previously grown for 3 days on T.B.A.B slopes at 30 ◦c in 10 ml sterile D.W, 0.1 ml of this inoculums was inoculated into a 250 ml conical flask containing 10 ml fresh Lubricating oil and 90 ml of mineral salt medium (M.S.M) , and incubated at 30◦c in rotary incubator for 7 days , 1 ml of the aqueous phase was taken at daily intervals to determine growth by colony counting on tryptose blood agar base (T.B.A.B.) (Korda ,et al. 2007 ,Brown & Braddock ,2001).

3-Growth of the isolates at different temperatures :-

Meat extract agar (M.E.A) and potato dextrose agar (P.D.A.) plates were inoculated with 0.1 ml of 18 hr. cell suspensions and incubated at 10,25,30,37,42, and 48 ◦c , the plates were examined daily for up to 7 days for the formation of visible colonies.

4-Growth of the isolates on oil at different temperatures :-

5 ml of Lubricating oil was added to 95 ml mineral salt medium (M.S.M.) in a flask inoculated with 0.1 ml cell suspension (3.6 X 10^6 cell\ ml) of one of each isolate , and incubated at 25,30,37,42, and 46 ◦c in a rotary incubator for 3 weeks, then the biomass and pH of the aqueous phase were determined (Korda , *et al.* 2007).

5- The effect of pH on the growth of the isolates:-

A different pH values of the M.S.M. of 2.1, 2.9 , 3.5 , 4.5 , 5.1 , 5.6 , 6.0 , 7.1 , 8.2 and 8.9 were prepared in flasks containing 100 ml of 5% v/v oil and inoculated with 0.1 ml cell suspension , the biomass was determined after 2 weeks incubation at 30 ◦c in rotary incubator(Korda ,*et al.* 2007).

6- Identification of the isolates:-

The isolates were identified according to standards methods, other methods have been consulted where appropriate (Stoke , 1971 , Van der walt , 1985).

7- The ability of the isolates to use the additives as a sole source of carbon :-

0.5 gm of each additive (i.e : anti rust , anti oxidant and gum inhibitor) was homogenized with 100 ml of M.S.M. , three flasks were prepared for each additive and each one inoculated with 0.1 ml cell suspension of either isolate, the flasks were incubated at 30 ◦c in a rotary incubator for 7 days , the growth was assayed by plate count method using M.E.A (Korda, *et al.* 2007 , Brown & Braddock ,2001).

RESULTS

1-Isolation and identification :-

the culture media which had been inoculated with the used oil and inoculated at 48 ◦c showed no signs of growth even after more than 7 days , this demonstrated that no thermophilic microorganisms had grown from these samples , but plates incubated at 30 ◦c produced different types of colonies and the isolates R2,R3,R5 were present in greater number than R1and R4,according to special keys for the genera of yeasts the strains coded R1,R2,R3, R4 and R5 were found to be *Candida maltose , Candida pulcherrina , Candida petrophilum , Candida albicans* and *Candida lipolytica* respectively (Stoke , 1971 , Van der walt , 1985).

2-Growth of the isolates on fresh oil

All the isolates were tested for their ability to grow in a two phase medium consisting of fresh oil plus M.S.M. as aqueous phase at 30°c , *C.petrophilum*, *C. lipolytica* and *C.pulcherrina* gave various degrees of growth (fig:1) , while *C.maltose* and *C.albicans* were unable to grow in this medium and after one day incubation no viable cells could be detected.

3-Growth of the isolates at different temperatures

Table(1) indicates the relative growth rate as determined by colonial appearance attained by the isolates at different temperatures after the time interval specified . At 48 ◦c all the isolates do not grow at all , however, although the growth density of *C.petrophilum* was greater at 10°c than that of *C. lipolytica* the latter exhibits more growth at 25 ◦c than *C.petrophilum* , of the temperatures chosen 30 °c proved to be the best for rapid growth of both isolates, *C.pulcherrina* showed no growth at 42 °c after 7 days incubation & the growth of it is slower than the others at other temperatures.

4- Biomass yield & temperature related

Data of the temperature related biomass yield after 3 weeks incubation of *C.petrophilum, C. lipolytica & C. pulcherrina* are shown with final pH of the aqueous phase in table (2) , the first two isolates were capable of growth over a wide range of temperature , while the third isolate exhibited a limited growth up to a temperature of about 37 °c , the maximum biomass were obtained at 25 °c

5- the effect of pH on the growth of the isolates

The biomass of the isolates grown at different initial pH levels were given in figure (2) , which shows that *C.petrophilum & C. lipolytica* grow equally well over a wide range of initial pH when grown on lubricating oils while *C. pulcherrina* showed a less growth rates , there was no significant growth at pH 2.1 in the culture of all isolates after 2 weeks incubation at 30 °c

6- Growth of the yeast isolates on oil additives

growth curves for *C. petrophilum & C. lipolytica* on antirust , antioxidant & gum inhibitor as a sole source of carbon are shown in figure (3) , the two isolates showed different growth characteristics , only slight growth occurred for the first isolate on antirust & anti oxidant , but it grow better on gum inhibitor, the second isolate showed a slight growth on gum inhibitor & anti oxidant , but greater growth on antirust , while *C. pulcherrina* showed a limited growth on all of the oil additives .

Discussion

It has been shown that of the organisms isolated from oil samples in this study, only three were capable of oil utilization , but none of these were bacteria could be due to the pH of the contaminating water , the effect of pH differ widely for various organisms ; fungi & yeasts grow more abundantly in acid environments .while most bacteria favour growth in nearly neutral environments (Blanch & Eiosele, 2003 , Erickson & Prokop, 2001) , since microorganisms depend on water for the synthesis of metabolites in general ; So chemical characteristics of the water phase are the dominant factor in determining whether growth occurs or n′t , even a trace of water is sufficient to initiate growth in petroleum product (Erdtsieck & Rietema, 2004 , Fukui & Tanaka, 1999) , thus lubricating oil systems must be contaminated with water to initiate microbial spoilage & the absence of moisture will prevent the infection (Erickson & Prokop, 2001) , the three isolates produce optimum growth at acidic pH &it was known that yeasts can grow better when the medium is acidic & results in figure (2) confirming this when grown well over a wide range of initial pH value of the water phase of the oil medium (Kappeli & Muller, 1998). It was found that the first

two yeast isolates can grow in medium with lubricating oil at a temperature of 42°c while *C.pulcherrina* was found to grow more slowly than the others & growth was limited above 37°c (table.1). *C. prtrophilum* & *C. lipolytica* are able to grow on ordinary microbiological media at a temperature of 42°c & higher (Fukui&Tanaka, 1999, Kappeli & Muller,1998 ,Mac Naughton, 1999), the ability of the isolated yeasts to grow on M.E.A. & P.D.A at 42°c confirming the pervious observation , several reports have been published which give results from studies on growth of yeast on hydrocarbon at elevated temperatures (Duppel,2002, Margesin& Schinner, 2001) , Orphan, et al. ,2002. Reported that Candida sp. was capable of growth up to 40°c on hydrocarbon based media), however Duppel(2002) stated that different strains of the same species of yeast may have different temperature tolerances (Duppel ,2002), the isolated yeasts were capable of metabolizing to varying degrees the pure additives, although it was difficult to compare the growth of the isolated strains grown on anti rust & gum inhibitors additives , but it demonstrates the ability for growth on such additives (fig. 3), the object was not to ascertain whether a particular oil additive is potentially biodegradable as a sole carbon source , but whether it was degraded by the organisms infecting the lubricating oils containing these additives , in general it appears from this study that microbial infection of lubricating oil not only results in the degradation of the basic oil components, but also in the degradation of the oil additives , this reduction in concentration of these additives in lubricating oil will sure affect it's performance level (Ijah ,2004 ,Oder ,2003) ,and the chemical composition of the oil will change & this will lead to an alteration of the physical properties of the oil itself (Van Hamme ,2000).

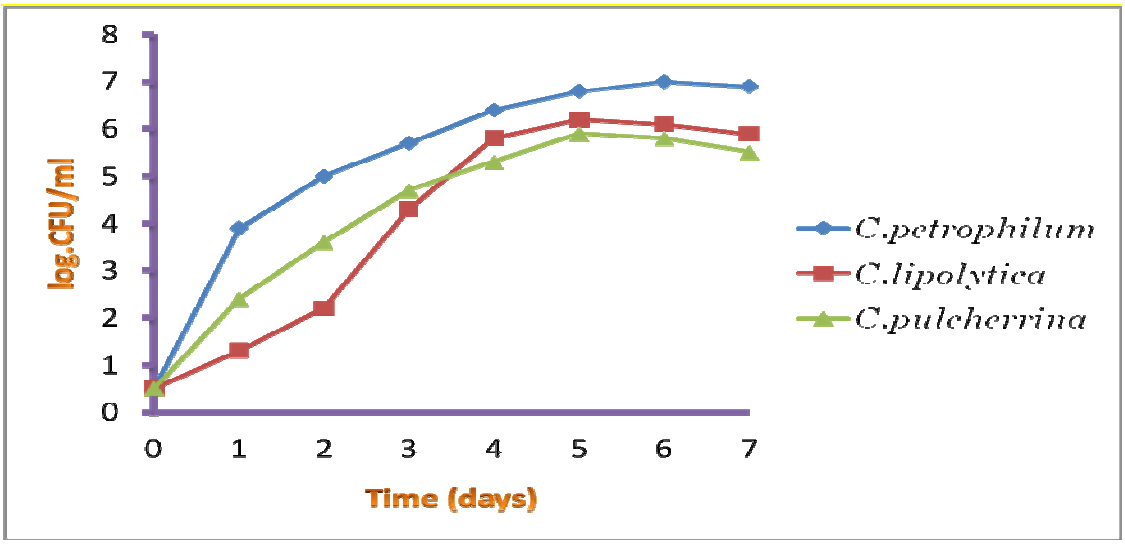


Figure (1) : growth of the isolated yeasts on fresh oil in M.S.M at 30 °c

Table (1): colony production of oil utilizing yeast on M.EA & P.D.A at different temperatures.

Incubation temperature	Isolates	Incubation period on M.E.A					Incubation period on P.D.A.				
		18h	24h	48h	72h	168h	18h	24h	48h	72h	
10 C	*C.petrophilum*	—	—	+	+	+	—	—	+	+	+
	C.lipolytica	—	—	—	—	+	—	—	—	—	+
	C.pulcherrina	—	—	—	—	+	—	—	—	—	+
25 C	As above	—	—	+	+	+	—	—	+	+	+
		—	+	+	+	+	—	+	+	+	+
		—	—	+	+	+	—	—	+	+	+
30 C	As above	+	+	+	+	+	+	+	+	+	+
		+	+	+	+	+	+	+	+	+	+
		—	—	+	+	+	—	—	+	+	+
37 C	As above	—	—	+	+	+	—	—	+	+	+
		—	—	+	+	+	—	—	+	+	+
		—	—	+	+	+	—	—	+	+	+
42 C	As above	—	—	+	+	+	—	—	+	+	+
		—	—	+	+	+	—	—	+	+	+
		—	—	—	—	—	—	—	—	—	—
48 C	As above	—	—	—	—	—	—	—	—	—	—
		—	—	—	—	—	—	—	—	—	—
		—	—	—	—	—	—	—	—	—	—

+ : visible colonies , — : no visible colonies

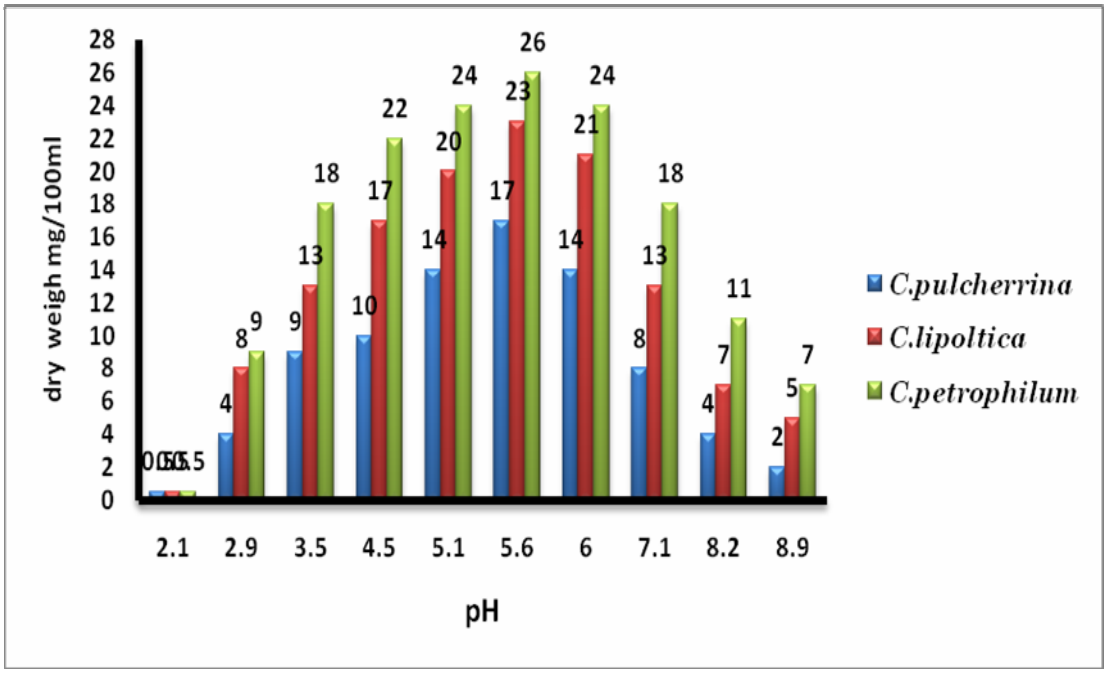
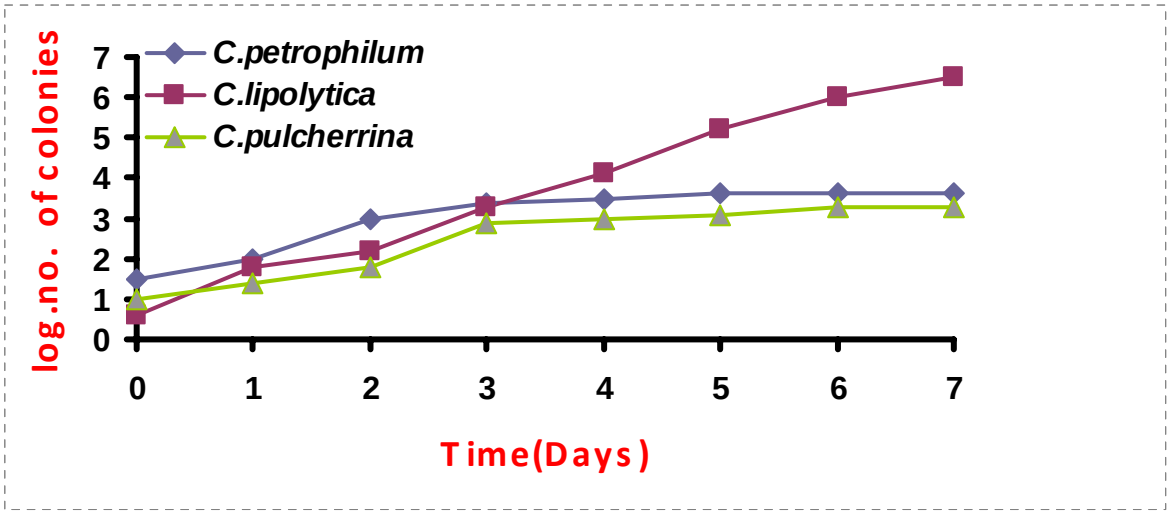


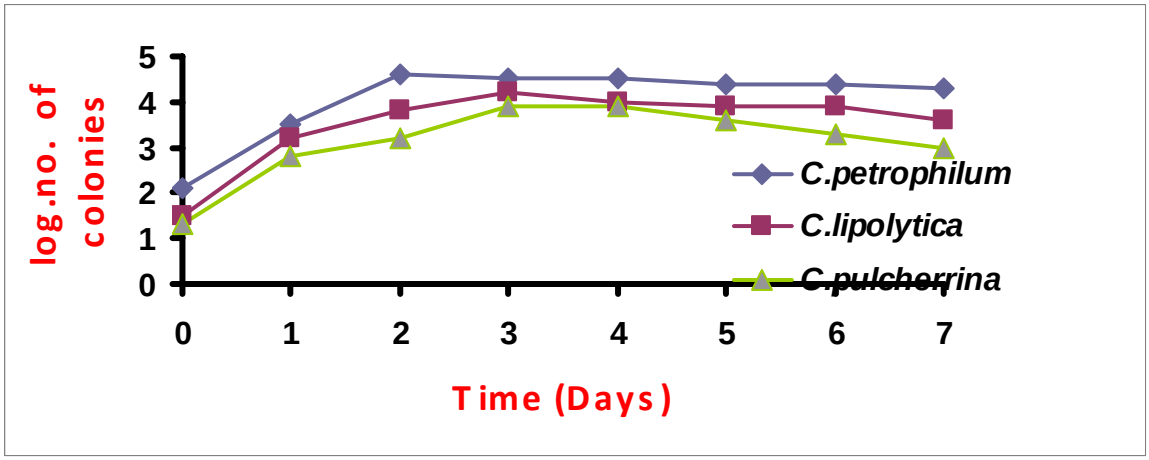
Figure (2) : cell yield of the yeasts on lubricating oil in M.S.M with different initial pH after 2week incubation at 30 ˚c .

Table (2) temperature related biomass & final pH of the isolates grown on oil in M.S.M after 3 week incubation.

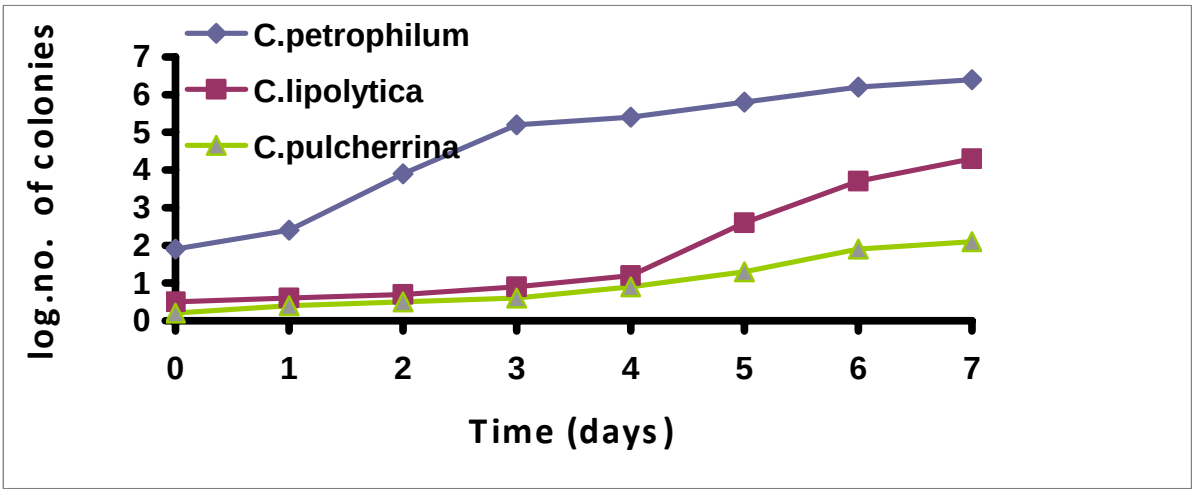
isolate / temperature	*C. petrophilum*		*C. lipolytica*		*C. pulcherrina*	
	Dry weight (mg/100 ml)	Final pH	Dry weight (mg/100 ml)	Final pH	Dry weight (mg/100 ml)	Final pH
25 C	104	3.7	76	4.4	63	6
30 C	73	4	60	4.7	37	6.3
37 C	42	5.8	25	5.8	9	6.5
42 C	16	6.2	11	6	2	6.6
46 C	2	6.6	2	6.5	1	6.7



(A) growth on antirust



(B) growth on antioxidant



(C) growth on gum inhibitor

Figure .(3): growth of the yeast isolates in 0.5% oil additives in M.S.M at 30 °c for 7 days .

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